

Figure 1

VH G250

D V K L V E S G G L V K L G G S L K L

1 GAC GTG AAG CTC GTG GAG TCT GGG GGA GGC TTA GTG AAG CTT GGA GGG TCC CTG AAA CTC

S C A A S G F T F S N Y Y M S W V R Q T
61 TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AAC TAT TAC ATG TCT TGG GTT CGC CAG ACT
H1

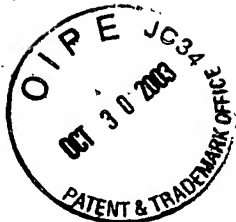
P E K R L E L V A A I N S D G G I T Y Y
121 CCA GAG AAG AGG CTG GAG TTG GTC GCA GCC ATT AAT AGT GAT GGT GGT ATC ACC TAC TAT
H2

L D T V K G R F T I S R D N A K N T L Y
181 CTA GAC ACT GTG AAG GGC CGA TTC ACC ATT TCA AGA GAC AAT GCC AAG AAC ACC CTG TAC

L Q M S S L K S E D T A L F Y C A R H R
241 CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC TTG TTT TAC TGT GCA AGA CAC CGC

S G Y F S M D Y W G Q G T S V T V S S
301 TCG GGC TAC TTT TCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA
H3

CDRs H1, H2, H3.
CDR definition according to Kabat scheme



VL G250

D I V M T Q S Q R F M S T T V G D R V S
1 GAC ATT GTG ATG ACC CAG TCT CAA AGA TTC ATG TCC ACA ACA GTA GGA GAC AGG GTC AGC

I T C K A S Q N V V S A V A W Y Q Q K P
61 ATC ACC TGC AAG GCC AGT CAG AAT GTG GTT TCT GCT GTT GCC TGG TAT CAA CAG AAA CCA

L1

G Q S P K L L I Y S A S N R Y T G V P D
121 GGA CAA TCT CCT AAA CTA CTG ATT TAC TCA GCA TCC AAT CGG TAC ACT GGA GTC CCT GAT

L2

R F T G S G S G T D F T L T I S N M Q S
181 CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATT AGC AAT ATG CAG TCT

E D L A D F F C Q Q Y S N Y P W T F G G
241 GAA GAC CTG GCT GAT TTT TTC TGT CAA CAA TAT AGC AAC TAT CCG TGG ACG TTC GGT GGA

L3

G T K L E I K
301 GGC ACC AAG CTG GAA ATC AAA

CDRs L1, L2, L3

CDR definition according to Kabat scheme

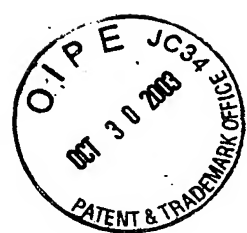


Figure 2

Primers used for PCR amplification of G250 VH and VL regions

5

Anchor and anchor poly C primers:

Anchor: 5'-GCA TGC GCG CGG CCG CGG AGG CC-3'

10

Anchor poly C: 5'-GCA TGC GCG CGG CCG CGG AGG CC(C)₁₂-3'

Constant primers:

15

VH-primers: 5'-CTC TAA GCT TGG CTC AAA CAC AGC
GAC CTC GGA TAC AGT TGG TGC AGC-3'

VL-primers: 5'-CTC TTC TAG AGA GTC TCT CAG CTG
GTA GGA TAC AGT TGG TGC AGC-3'



Figure 3A

08.05.2001

double strand sequencing

HC_cG250 (EcoRI-fragment in expression vector)

1 TCATGACATT AACCTATMAA AATAGGCCGTA TCACGAGGCC CTTTCGTCTT CAAGAATCT TCAGATACAA AGAATCTCTA AACCTGAGG ACATTCTATC 100
101 ACAATAAGT AAAATTCAGA AAATCTGAA TGCCTCCATC ACAGAGATGA ATCTGCTATG AACAGCTCAT AGGTGTGAAG CTCTACAAA GCCATATTAT 200
201 TGAAAAGCCA CATTGTGCC AGACTTTGGA AAGACTGAGC TCATATCCTG AAATACAGTT ATGTGTGGTT CTAATCTAAT ACACATTTAC ACTAAGGAAA 300
301 CATGGCAGTA TGGGAATGAA GCTTGTCTG TACACATTA CAGAGGGAAA CTAACACAAAG TATGGTGAAT CCTAACCAA AAGTAAAAA AAAAAAAAA 400
401 AAGAAAGAA AAGAAAAAA AAGTGAAC AATATATGTT TCAAAATGCTG TAACTGAAT CTGGTTTTT GATGCCCTAT ATCTGTATC ATCAGTGACT 500
501 TCAGATTAG TCCAACCTCA GAGCATGTA TAGCAGGAAG ACATGCNAAT AGGTCTTCTC TGTGCCCATG AAAACACACCT CGGCCCTGAC CCTGCAGCTC 600
601 TGACAGAGGA GGCCTGTCTT GGATTCGATT CCCAGTTCTT CACATTCAGT GATCAGCACT GAACACAGAC CCTCACCAT GAACCTCGG CTCAGANTGA 700
701 TTTTCTTGT CTTGTGTTTA AAGGTATCT TATTGATAT AGAGACATC TGCTGTATGC ACAGAGTGC AGAAAAATG TTTTGTGTT TTTTGTGTA 800
801 CAATGCTCA AACAGTATC TTTCTTTGCA GGTGTCTCTT GTGACGTGAA GCTCGTGGAG TCTGGGGAG GCTTAGTGA GCITGGAGG TCCCTGAAAC 900
901 TCTCTGTGC AGCTCTGGA TTCACTTTCA GTAACATTA CATGTCTTGG GTTCGCCAGA CTCCAGAGAA GAGGTGGAG TTGTCGCAG CCATTAATAG 1000
1001 TGATGGTGGT ATCACCCTACT ATCTAGACAC TGTGAAGGC CGATTACCA TTTCAGAGA CAATGCCAAG AACACCTCT ACCTGCAAC CTCTCCTCAG 1200
1101 AAGCTGAGG ACACAGCCCT GTTTTACTGT GCAGACACC GCTCGGGCTA CTTTCTATG GACTACTGG GTCAGGAACT CTCAGTCACC GTCTCCTCAG 1300
1201 GTAAGAAATGG CCTCTCCAGG TCTTTTTTTT AATCTTTGTA ATGGAGTTTT CTGAACATG CAGACTAATC TTGGATATTT GTCCCTGAGG TAGCCGGCTG 1400
1301 AGAGAAATG GGAATTAAC TGCTCGGGA TCTCAGAGCC TTTAGGACAG ATTATCTCCA CATCTTTGAA AACTGAGAT TCTGTGTGAT GGTGTTGGTG 1500
1401 GAGTCCCTGG ATGATGGGAT AGGACTTTG GAGGCTCANT TGAGGAGAT GCTAAACAA TCCTATGGCT GGAGGAGAG TTGGGGCTGT AGTTGGAGAT 1600
1501 TTTTCAGTTT TAGAATAAAA GCTTTAGCTG CGGGAATCC TTCAGGACCA CCTCTGTGAC AGCATTTATA CAGTATCCGA TGCATAGGGA CAAAGAGTGG 1700
1601 AGTGGGCAC TTTCTTTGGA TTTGTGGGA ATGTTCCACA CTAGTTTCTG TGAACCTCA TTTGTGGAG GGAGAGCTGT CTTAGTGCCT GAGTCAAGG 1800
1701 AGRAGGGCAT CTAGCCTCGG TCTCAAAAG GTAGTTGCTG TCCAGAGAGG TCTGGTGGAG CCTGCAAAAG TCCAGCTTC AAGGAACAC AGAAGTATGT 1900
1801 GTATGGAATA ATAGAAGATG TTGCTTTTAC TCTTAAGTTG GTTCATAGGA AAAATAGTTA AACTGTGAG TTTAAAAATG GAGAGGGTTT TCAAGTACTC 2000
1901 ATTTTCTTAC ATGTCCAAA TTTCTGTCAA TCAATTTGAG GTCTGTGTTG TGTAGAACTG ACATTTACTTA AAGTTAACC GAGGAATGG AGTGAGGCTC 2100
2001 TCTCATACCC, TATTCAGAAC TGACTTTTAA CAAATATAA TTAAGTTTAA AATTTTTTA AATGATTA GCAATGTTGA GTTGGAGTCA AGATGCCGA 2100



10.05.2001

double strand sequencing

LC_cG250 (HindIII-fragment in expression vector)

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4501 GAGATCTGGG TCTGACTGCA GGTAGCGTGG TCTTCTAGAC GTTTAAGTGG GAGATTTGGG GGGGATGAGG AATGAAGGAA CTTCAAGGNTA GAAAAAGGTCT 4600
4601 GAAGTCAAGT TCAGCTCCTA AATGGATGT GGGAGCAAAAC TTTGAGAGATA AACTGAATGA CCCAGAGGAT GAACAGTGC AGATCAAAAGA GGGGCTTGA 4700
4701 GCTCTGAGAA CAGAAGGAGA GTCATTCGTG TTGAGTTTCC ACAAACTACTG TCTTGAGTTT TGCAATAAAA GTGGGATAGC AGAGTTGAGT GAGCCATAGG 4800
4801 CTGAGTCTC TCTTTTGCT CTTAAGTTT TATGACTACA AAATCAGTA GTATGTCTCTG AAATAATCAT TAACTGTGT GAAAGTATGA CTGCTTGCCA 4900
4901 TGTAGATACC ATGGCTTGCT GAATAATCAG AAGAGGTGTG ACTCTTATTC TAAAAATTTGT CACAAAATGT CAAAATGAGA GACTCTGTAG GAACGAGTCC 5000
5001 TTGACAGACA GCTCAAGGGG TTTTTCCT TTGTCCTCAT TCTACATGAA AGTAAATTTG AAATGATCTT TTTTATTATA ATAGTAGAAA TACAGTTGGG 5100
5101 TTTGAACTAT ATGTTTAAAT GGCCACGGTT TTGTAAGACA TTTGGCCCTT TGTTTTCCCA GTTATTACTC GCTTGTAAAT TTTATATCGCC AGCAATGGAC 5200
5201 TGAACGGTC CGCAACCTCT TCTTTACAAC TGGGTGACCT CGCGGCTGTG CCAGCCATTT GGCCTTCACC TTGCCGTAA GGGCCGTGTG AACCCCGAG 5300
5301 GTAGCATCCC TTGCTCCGG TGGACCACTT TCCTGAGGCA CAGTGATAGG AACAGAGCCA CTAATCTGAA GAGAACAGAG ATGTGACAGA CTACACTAAT 5400
5401 GTTAGAAAAA CAAGGAAGG GTGACTTATT GGAGATTTC GAAATAAAT GCATTTATTA TTATATTCCC TTATTTTAAAT TTTCTATTAG GGAATTAGAA 5500
5501 AGGCATAAA CTGCTTTATC CAGTGTATA TTAAGAAGCTT TTTTTCCTC AGTGCTA 5557
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Figure 3B:

10.05.2001

double strand sequencing

LC_cG250 (HindIII-fragment in expression vector)

1	AATTCGAAGC	TTTGTATCTT	CAGATCCAGG	AAAGCCACCA	CCAAATCAAA	ACAGATACAT	GCTGAAGCCA	ACTTCTGTTT	TTATGTCAAA	TGCACAGCGG	100
101	GCATCTGACA	CTGCCCTGCAT	GAAGTCTCA	GGTCAANTACT	TCCACTACAC	ACATGGAAGC	TGACACCCAAT	GAGTCAATA	TTTAGTCTTT	TTGCCCATTT	200
201	CAGGAGGAGA	CTGCTGGTTT	TGAGTGTGGC	ACCAGACTTA	ACACCAAGTC	GACAAACTGC	TTTGGAAATCA	TCTGTGACAA	TCCACAAAAA	CNACTTTGTC	300
301	TTACAANTGT	CTCTGACGAC	ATTCAATCAAT	TCAATTTCACT	GTCAAAAAGTC	ATCATCTGGA	CTCCATTTACT	GGCAGCATAC	TTGAATTTGAG	ACACTTTGTT	400
401	ACAAAAAATGT	GCATAGGTAA	TCCTCTCTGG	AGGAACCCAGA	AGCCCCCGTT	CCAACTGTAT	TTTCACTCTTG	CTTGCACAGT	CAANTCCTGT	ACCAATAGCA	500
501	GCTAGGGTGT	TAACTATGGC	TCTGTTGTCC	TTACACTTTGA	CTGCACAAAA	AGGAATAACA	TTTGGGAAGAG	CTTTTAGCCA	CCTCAGATGC	TTCTTTTAGAA	600
601	TGCTCTCTGAG	GTCCGGAACC	TAGAAAGAAG	AGACTTCAAT	TATTAATTTTG	TGTTTCAGAA	GTCTTTAGCA	CTAAAGCCAC	CATCTATGAT	ACAGCAGTCA	700
701	AACTCTTCTCT	TAGTATAGCT	GCTCATCGTT	CTCCATGTGC	CTACAGAAAA	CCTAGACATG	GAATTAATTT	ATTGCCAGCC	CCTTACAAGG	TCAACTTATC	800
801	CAAGAACTGT	GAATGCAGAC	TCCTTGAANT	GTGGGAACA	CTCACAGCAC	AGGTCAAGA	CTGGCTGGAC	ACATGGAGAC	ACTGAATCCT	GAAGAGCACT	900
901	TAGCTGTCTG	TTGCTTCATC	ATGCTACTG	ACCTGAGGTG	GCACCAAGCT	GCTTACTGAG	GGAGGACTGT	GGCGGTGCT	GCAGGAACCTG	ACAATTTCTC	1000
1001	ACAATTTCTCT	TACTGCCCCA	CTCATAACTC	TTCTCTTCTC	CATCTTCTTC	TTTCTTTTCT	CTTCCCTCCT	TTTTCCCTTT	CACACTTTTT	TTCTTTTCTT	1100
1101	CTTTTCCACT	TCCCTTTTCT	TTCTTCTTTT	GCTGTGTCTG	TTGTAAAGGA	TTTATTTGTT	CCCTGTGATT	GAACCAAGG	TAGTTGTACT	ATTATTTCTG	1200
1201	TAAAACTCAT	CTGTGTGATT	TCTATTAAT	AATTAATTTT	GTTTACACTC	CATATTTTAT	TCAACCCCTC	CATCTCCTA	CTGCTCTACA	TACCATACCT	1300
1301	CTTTCCACCA	CCCCGTGCTC	CACATGGATG	CTGCCACCTC	CCATGCCACC	TGACCTCTCA	TCTCCCTAGG	GCATCTAGTC	TCTTGAGGCT	TAGATGCATC	1400
1401	ATTTCTGAGT	GAACACAGAT	CCAACAATCC	TCTGTATAT	GTGTGTGTGT	GGCCTCATAG	CAGCTGGTGT	ATGCTGCCCTG	TTTGTGTGATC	CAGTGTTTGA	1500
1501	GAGGTCTCGC	GGGTTTCAGAT	TAAATGAGAT	TGTTGGACCT	CCTCAGCGTC	TTTTCAGTCT	TCCCTGATTC	AACAACAGGG	TTTCAATTTT	CTGTTCATTTG	1600
1601	GTGGGTGCA	AATATCTGCA	TCTGACTCAG	CTGCTTATTG	GGTCTTCTGG	AGTGCAGTCA	TGCTAGGTCC	GTTCCTATGA	GTGCTCCATA	GCCTCAGTGA	1700
1701	TAGTGTGAGG	CGTTGGGACT	GCCCCCTTGC	CTGGATTTCTA	TTTTGGACCT	GTGCTGGGAC	CTTCTTTTCC	TCAGGCTCCC	CTCCATCTGT	ATCCCTGTAA	1800
1801	TTCTTTTACA	CAGGAACAAA	TATGGGTACAG	AGTTGTGAGT	GTGGAATGGC	ACCCCTTCTC	CTCATTTAAT	GGCCTGTCTT	CCTGGTGGAA	GTGGGCTCTA	1900
1901	TAACTTCCCA	CTCCCTACTG	TTGGGCAATTT	CATCCCTTTG	AGTCTTGAGA	GTCTCTCACC	TCCAGGCTCT	CTGTGTCAAT	CTGGAGGGTC	CTCCCACTCT	2000
2001	CTTACCTCCC	CAGTTGCCT	GTTCACAGAC	TTCTGTCTGGC	CCCGATGCT	TCAGTCTCTT	TCCCTCACCC	AATATCTGAT	TTGGATGGAA	GCCTGTCTATG	2100



HC_cG250 (EcoRI-fragment in expression vector) double strand sequencing 08.05.2001

2101 TCAGAACCCAG AACACCTGCA GCAGCTGGCA GGAAGCAGGT CATGTGGCAA GGCTATTGG GGAAGGGAAA ATAAACCAC TAGGTAACT TGTAGCTGTG 2200

2201 GTTTGAAGAA GTGGTTTTGA AACACTCTGT CCAGCCCCAC CAAACCGAAA GTCCAGGCTG AGCAAAACAC CACCTGGGTG ATTTGCATTT CTAAATAAAG 2300

2301 TTGAGGATTC AGCCGAAACT GGAGAGGTCC TCCTTTAACT TATTGAGTTC AACCTTTTAA TTTTACCTTG AGTAGTCTA GTTCCCCCA ACTTAAGTTT 2400

2401 ATCGACTTCT AAATGTATT TAGAATTCTAT T 2431



LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

2101 AGAACATCTA TATACCTG GTTTCAGAGC TTAAATATGG TCCTTGAGCT TCTATTTTGA GTTCCTTTCC AGTGATTACT TGCCTGCTTTT GGTAGTACTT 2200
2201 TTGACTGTTT ATTAAACCTG GATACTCTCA TACAGCTGTG TAATTTACTT CCTTATTTGA TGAATGCTTT GCATAGATCC CTAGAGGCCA GCCCAGCTGC 2300
2301 CCATGATTTA TAAACCAGGT CTTTGCACTG AGATCTGAAA TACATCAGAA CAGCATGGGC TTCAAGATGG AGTTTCATAC TCAGGTCTTT GTATTCGTGT 2400
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2601 GAGACATTTG GATGACCCAG TCTCAAAAGAT TCAATGTCAC AACAGTAGGA GACAGGGTCA GCATCACCTG CAAGGCCAGT CAGATGTGG TTCTTGCTGT 2700
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4001 TCCTGGCAAC CTGTGCATCA ATAGAAGATC CCCCAGAAA GAGTCAGTGT GAAAGCTGAG CGAAAACCTC GTCATTAGCT TCTGAGACCA GTTTTGTAA 4100
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4301 TAGGAGTTAA AGTCAGTTCA GAAATCTTG AGAAAATGGA GAGGGCTCAT TATCAGTTGA CGTGGCATAC AGTGTGAGAT TTTCTGTTTA TCAAGCTAGT 4400
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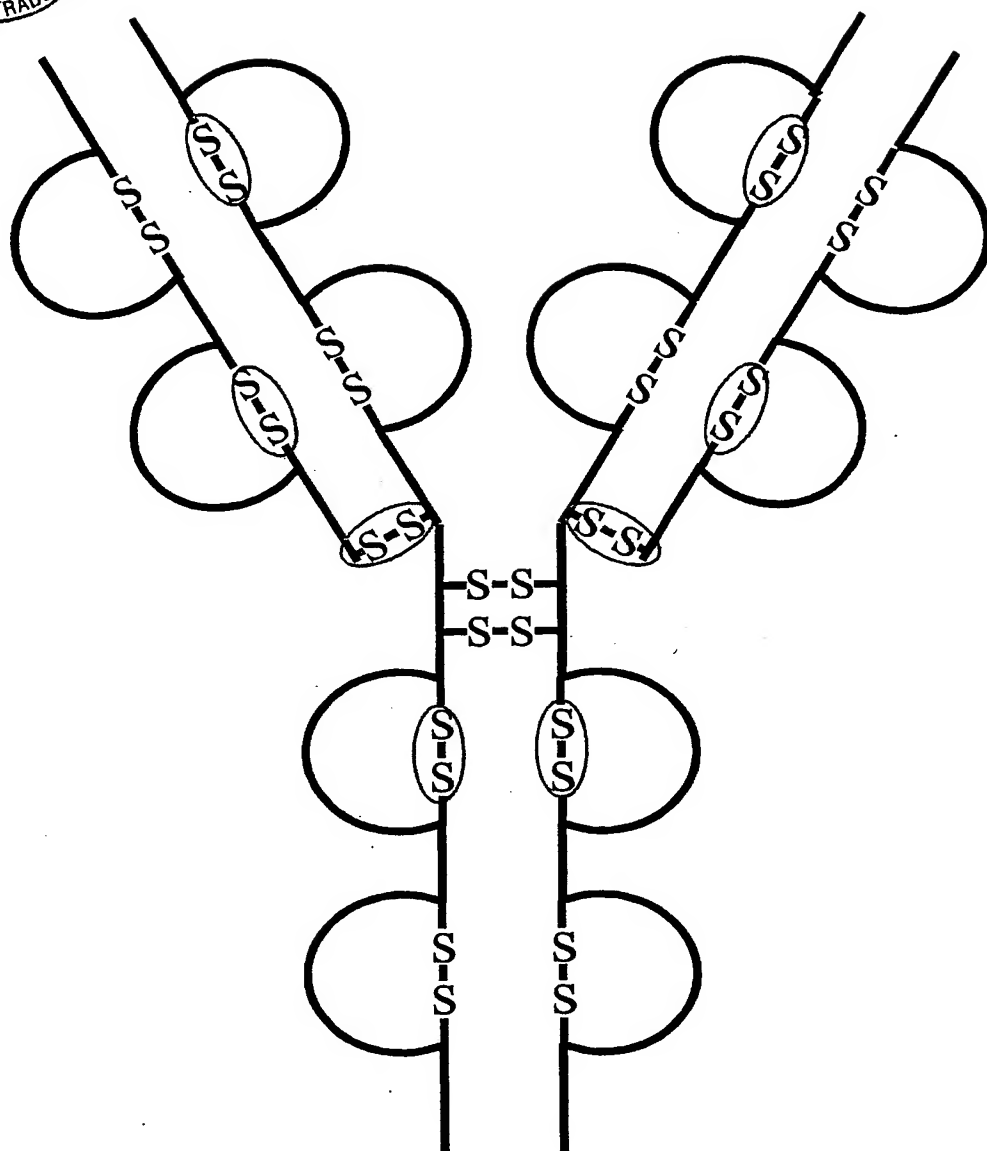


Figure 4

Schematic figure of an antibody. Disulfide bonds detected by mass spectrometry after enzymatic digest in WX-G250 are labeled by ovals.

Figure 5

Characterization of WX-G250 according to MALDI-PMF (Trypsin, LysC, AspN, GluC, and BrCN) in reflector and linear mod



Light Chain:

DIVMTQSQR F MSTTVGDRVS IT C KASQNVV SAVAWYQQKP

GQSPKLLIYS ASNRYTGVPD RFTGSGSGTD FTLTISNMQS

EDLADFE C QQ YSNYPWTEGG GTKLEIKRTV AAPSVFIFPP

SDEQLKSGTA SVV C LLNNFY PREAKVQWKV DNALQSGNSQ

ESVTEQDSKD STYSLSSTLT LSKADYKHK VYA C EVTHQG

LSSPVTKSFN RGE C

Heavy Chain:

DVKLVESGGG LVKLGGSLKL S C AASGFTES NYMSWVRQT

PEKRLELVAA INSDGGITYY LDTVKGRTI SRDNAKNTLY

LQMSSLKSED TALFY C ARHR SGYFSMDYWG QGTSVTVSSA

STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW

NSGALTSGVH TFAVLQSSG LYSLSVTV PSSSLGTQTY

I C NVNHKPSN TKVDKKVEPK SCDKTH C PP CPAPELLGGP

SVLEFPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY

VDGVEVHNAK TKPREEQYNS C TYRVVSVLTV LHQDWLNGKE

YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL

TKNQVSLT C L VKGFYPSDIA VEWESNGQPE NNYKTTPVL

DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ

KSLSLSPG C K

----- Reflector mode
----- Additional information
----- from linear mode spectra

C Detected bridged cysteins (reflector mode)
C Detected bridged cysteins (linear mode)
C Cystein not determined as bridged cysteins
C Heavy chain partially lacks C-terminal lysine



Confirmed glycosylation site



Confirmed deamidation sites



Light chain:

DIVMTQSQRF MSTTVGDRVS ITCKASQNVV SAVAWYQOKP GQSPKLLIYS
ASNRYTGVPD RFTGSGSGTD FTLTISNMQS EDLADFFCQQ YSNYPWTFGG
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

Heavy chain:

DVKLVESGGG LVKLGGSLKL SCAASGFTFS NYMSWVRQT PEKRLELVAA
INSDGGITYY LDTVKGRTI SRDNAKNTLY LQMSSLKSED TALFYCARHR
SGYFSMDYWG QGTSVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

Figure 6: LC-MS and LC-MS/MS of tryptic digest of cG250